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PATHOMORPHOLOGICAL AND HISTOCHEMICAL CHANGES IN THE ORGANS OF TURTLES ON BOARD THE AUTOMATIC STATION "ZOND-5"

by

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EDITED TRANSLATION

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PATHOMORPHOLOGICAL AND HISTOCHEMICAL CHANGES IN THE ORGANS OF TURTLES ON BOARD THE AUTOMATIC STATION "ZOND-5"

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For a number of reasons certain reptiles, in particular turtles, are suitable for carrying out some biological research in space. The level of organization of these animals is not greatly below that of mammals; along with this, they do not require complex special systems of life support and they can be harnessed rigidly on board space vehicles. With seasonal distinctions taken into account, the animals are available for study at any time of year. On the basis of these general considerations, the program of biological research in space has included experiments with turtles.

Along with other biological objects, the automatic station "Zond-5," which accomplished a flight around the Moon, carried steppe turtles (*Testudo horsefieldi gray*). A total of 8 pubescent turtles - males 6-7 years of age weighing 340-400 g - were studied. Two animals (experimental group) were placed on the automatic station (Fig. 1); two (control group) were transported to the cosmodrome and back; four turtles (undisturbed group) were retained in the vivarium.

The animals were delivered to the laboratory two months before the beginning of the experiment. In the course of the two months

the turtles were weighted repeatedly, studies were made of their peripheral blood, and EKG were taken at three standard leadoffs. Besides this, detailed observations were made of the eating activity of the animals. The daily ration of the turtles consisted of meat (2 g), cabbage (10 g), carrots (10 g), and bread (10 g).

On the automatic station the animals of the experimental group were placed in snug individual cages, in which they were practically unable to move. Cages of the same type were used for the control animals. The turtles of the experimental and control groups received no food or water over the course of the entire experiment, while the undisturbed animals were placed in free cages and fed their usual ration.

The turtles were placed on board the "Zond-5" station on 2 September 1968. From this time they were not fed. The launch took place, as is known, on 15 September. After circling the Moon and returning to the Earth the station splashed down in the Indian Ocean on 21 September. The object was delivered to Bombay on 3 October and on 7 October it was returned to Moscow. Pathomorphological investigation of the turtles was conducted on 11 October.

As is evident from the sequence of experiments which is given here, the experimental animals were subjected to a 39-day fast, to spaceflight factors continuing for about 7 days, to the action of a tropical climate, and to conditions connected with a sojourn on the ocean after splashdown and with transportation on a ship and in an airplane. According to data from dosimetry carried out on the station, the total dose of radiation received by the experimental animals did not exceed 3.5 rad.

The animals in the control group not only were not subjected to factors due to spaceflight, but were also spared the conditions of a tropical climate and the additional transportation.

The biological effect on the animals of the complex of flight factors and the other conditions arising during the experiment

was evaluated by means of several hematological tests, electrocardiography, and a number of pathomorphological and histochemical research procedures. Electrocardiography was conducted prior to beginning of the experiment and after the flight around the Moon and delivery of the animals to the laboratory.

When the animals were sacrificed, an original, specially developed procedure was used to make tissue blocks from pieces of the intestines, spleen, testes, seminal vesicles, liver, kidneys, and heart for subsequent histological and histochemical study.

The principle of the method of combined tissue blocks is as follows: an organ taken from an animal of the experimental group is glued on filter paper together with the same organs removed from animals of the control and undisturbed groups. Tissue blocks prepared in this way are frozen in dry ice and combined sections are prepared in a cryostat for histochemical studies. Analogous blocks, not frozen, are placed in fixing mixtures and are passed through dehydrating and condensing media to obtain sections suitable for processing by histological procedures. Sections were prepared on a microtome by the usual procedures, using combined blocks fixed in paraffin and dyed according to appropriate procedures. Thus, we obtained combined sections from the organs of different animals on a single slide, sections with identical thickness and prepared under identical conditions. This permitted reliable comparison of structural changes in the organs of the animals in the experimental, control, and undisturbed groups. The sections were stained with hematoxylin-eosin, RNA was demonstrated by methyl green – pyronine after Brashe, glycogen was stained per Shabadash, lipids were stained with red scarlet, and iron was demonstrated with Perls stain. Determinations were also made of the activity of succinate-dehydrogenase (SDH) per Nakhlas et al., of monoaminooxidase (MAO) per Glenner, alkaline phosphatase (AP) per Gomori (E. Pierce, 1962), and of the activity of α-glycerophosphate dehydrogenase (α-GPD) after D. Quadlino et al. (1960) in a modification by R. P. Nartsissov (1968).

The animals of all three groups displayed no distinctions in external appearance and behavior at the time of examination.

The level of feeding activity was identically high in the animals of the experimental and control groups, carried to the cosmodrome, and in the undisturbed group located in the vivarium. The weight of the turtles which were carried on the flight was reduced in comparison with the initial, preflight weight by about 10%. The weight of the turtles in the control group was reduced by only 5%. No significant distinctions in peripheral blood of the examined turtles were detected.

Analysis of electrocardiograms recorded at various periods before and after the flight did not manifest any particular changes in cardiac activity of either experimental or control animals. According to our data the frequency of cardiac contractions for the turtles in the active state in the vivarium varied from 14 to 48 contractions per minute. A well-expressed arrhythmia was characteristic for turtles; it is clearly of vagus origin. In our observations the R-R interval varied from 20 to 49 seconds. During painful stimulation (the point of a needle in the sural muscle) quickening of cardiac contractions by 10-12 beats per minute appeared. Return of the pulse frequency to the initial level set in relatively quickly – within 1-2 minutes.

Table 1 and Fig. 2 show results of investigation from recording of an EKG on the twenty-first day after completion of the flight.

As is evident from the table and the EKG, no essential distinctions were detected between the experimental and control animal with respect to a number of studied indices (frequency of cardiac contractions, R-R interval). As regards changes with respect to individual waves of the EKG, owing to insufficient data it is impossible to draw any definite conclusions.

Table 1. Characteristics of cardiac activity of turtles flown on the automatic station "Zond-5."

P. No.	Animal No.		Frequency of cardiac contractions per minute	R-R interval in seconds. Drawing rate 15 mm/s.
I	№ 22	Experimental	28	30-49
2	№ 37	Experimental	28	32-34
3	№ 49	Control, flown to the cosmodrome	32	29-31
4	№ 47	Control, in vivarium	30	22-23

During macroscopic study of the internal organs it was found that in turtles of the experimental and control group the thickness of the intestines was reduced over the entire length. In the experimental animals the surface of the liver was an intensely brown color, while in undisturbed turtles it was a dark cherry and in the control turtles the color of the liver surface occupied an intermediate position.

Significant distinctions were found during comparative microscopic study of the organs of experimental and undisturbed turtles. In the experimental animals the diameter of the intestine and the thickness of the muscular layer were reduced; the villi were shortened in places (Fig. 3). The mitotic activity of the epithelium of the crypt was suppressed. Some epithelial cells had pyknotic nuclei (Fig. 4). Clots of brown pigment appeared in the cytoplasm of cells of the villi stroma and of some epithelial cells; this was not observed in the undisturbed animals. The content of PAS-positive substances was reduced (Fig. 5). The number of goblet cells was reduced, particularly in the depth of the crypt; the activity of MAO and α-GPD (and partially, of AP) in the epithelial cells of the crypt and also in the nerve cells in the Meissner and Auerbach plexi was lowered. The activity of SDH in these cellular elements was somewhat increased. The follicles of the spleen

were reduced and were completely without mitosis figures. Some lymphocytes had pyknotic nuclei, something which was not observed in the undisturbed animals.

In the testes the convoluted tubules were reduced in diameter (Figs. 6 and 7); brown pigment had accumulated in the interstitial tissues. In the undisturbed animals it appeared only in individual cells. This pigment was also detected in many retained germ cells; on undyed preparations it had a dark-orange color and gave a negative reaction on iron moderately stained with pigment brown. The conglomerates of pigment possessed birefringence. The number of germ cells and the concentration of RNA in them were significantly reduced (Figs. 6 and 7); the activity of SDH was somewhat increased, while that of MAO and α-GPD was reduced. The seminal vesicles of the experimental turtles were empty (Fig. 8), while in the undisturbed animals their lumen was filled with spermatozoids (Fig. 9). The concentration of RNA and the activity of enzymes in the seminal vesicles was changed as compared with the undisturbed control in the same way as in the testes.

In the liver a reduction in the size of hepatocytes and their nuclei was observed. The cytoplasm of the cells had become basophilic (Fig. 10). In the places in which endothelial-reticular cells had accumulated there was an increase in the quantity of brown clotlike pigment, and in the lumina of the biliary capillaries and in the cytoplasm of the hepatocytes there were powdery granules of trivalent iron. In the cells of the liver RNA was found in the nucleoli and, in small amounts, in the perinuclear zone. The concentration of RNA was not great, but it was higher than in the undisturbed animals. The content of glycogen was also increased (Figs. 11 and 12); this was demonstrated not only in hepatocytes, but also in the cells of the peripheral blood – mainly in the leucocytes and in free reticular cells located in the lumina of the vessels. Fat had disappeared from the cells of the liver, while in undisturbed animals it appeared in a significant quantity (Fig. 13). In the livers of the undisturbed turtles there were almost no deposits of granules of formazan,

corresponding to activity of SDH, while in the experimental animals they appeared in some hepatocytes, in many Kupffer cells, in the walls of the vessels, and in the bile ducts; the activity of AP was also increased. On the other hand, the activity of MAO in the hepatocytes was distinctly reduced (Fig. 14), and that in elements of the peripheral blood was completely suppressed.

In the kidney the volume of nuclei of epithelial cells of the convoluted tubules was reduced, some nuclei had become pyknotic, the outlines of the cells were irregular; grains of brown pigment sometimes appeared in the cytoplasm of the cells. This pigment gave no reaction to iron and was not stained by pigment stain. Pulverulent granules of iron-containing pigment were manifested. Along with this, the RNA in the nucleoli was manifested more weakly in the cells of the epithelium of many convoluted tubules. The activity of SDH and, to a lesser degree, that of AP was increased, while the activity of both MAO and α-GPD was reduced.

No changes appeared in the hearts of the experimental animals.

In turtles of the control group, which were delivered to the point from which the automatic station was launched and subjected only to the action of the weather, we noticed changes which were the same in character as those in the animals of the experimental group. In some organs the degree of the changes was less expressed. In the turtles of the control group cells with hyperchromic nuclei were smaller in the epithelium of the intestinal crypt; in one turtle individual mitoses were manifested in the crypts. Mitotic activity of cells was noted also in the follicles of the spleen, which were reduced as compared with the spleen follicles of the undisturbed animals but were nonetheless somewhat larger than those in the experimental turtles. The changes in the structure of the testes were the same as those in the experimental animals, i.e., the size of the tubules and the number of germ cells were reduced and an increase was observed in the lipofuscin in the interstitial tissue. In contrast to the experimental turtles, in the seminal

vesicles of the control animals spermatozoids were found (Fig. 15). Along with this, there was little change in the activity of SDH, AP, and α-GPD in the testes and the walls of the seminal vesicles. Less expressed changes in enzyme activity, especially of MAO, was observed also in the liver.

Thus, in the turtles of the experimental and control groups there was a reduction in the diameter of the intestine, the thickness of the tunica muscularis, and the length of villi. Cells with pyknotic nuclei and inclusions of lipofuscin appeared in the epithelium of the crypts; the number of basophilic cells was reduced. All of this indicates development of atrophy of the intestine caused by fasting. Its functional activity was reduced, as indicated by the suppression of mitotic activity of the epithelium of the crypt and by the reduction in RNA concentration in the epithelial cells of the mucous membrane.

Fasting and dehydration of the organism caused changes of an atrophic character in other organs: disappearance of lipids from the liver, a reduction in the size and volume of cells of the liver and the kidneys, accumulation of lipofuscin in these organs and, especially, in the interstitial tissue of the testes, reduction in the diameter of the seminiferous tubules and in the number of germ cells of the spermatogenic epithelium, and disappearance of spermatozoids from the seminal vesicles. A certain increase in RNA concentration and in the content of glycogen in liver cells is connected, in our opinion, with a reduction in the volume of cells and with their dehydration.

The increased iron content in the liver and kidneys may possibly be connected with haemolysis of erythrocites and also with a reduction in the amount of iron required for haematopoiesis, which is suppressed during starvation.

Atrophy caused a shift in the enzyme activity of tissues: in the walls of the intestine, in the testes, liver, and kidneys the activity of SDH grew considerably and that of AP increased to

a lesser degree, while the activity of MAO and α-GPD was reduced.

However, all of the enumerated changes in the organs of the turtles cannot be explained by the action of starvation alone. The fact is that in the turtles of the control group, who were starved right along with the experimental animals, the changes in the organs were less expressed. Thus pyknotic cells were encountered very rarely in the epithelium of the intestinal crypt in control animals and individual mitosis figures were encountered. Spermatozoids were observed in the seminal vesicles in a quantity almost exactly the same as that in the undisturbed turtles. The reduction in the volume of follicles was less expressed. Mitotically active cells were detected in some follicles. Enzyme activity in the tissues was also less sharply changed.

Apparently, this distinction should be explained by the additional action of spaceflight factors. In particular this may be indicated by the higher level of reduction in MAO activity in the organs of the experimental animals. As is known, monoamino-oxidase is a self-oxidizing enzyme, participating in the regulation of the volume of biologically active compounds, including serotonin. Under the influence of individual flight factors such as g-force, vibration, and penetrating radiation, the level of serotonin in the blood changes (V. V. Parin et al., 1964-65; V. V. Antipov et al., 1967).

If ecological peculiarities are taken into account, including the sharply expressed seasonal variation in physiological activity (sharply increased in turtles during the warm season), we cannot exclude also the effect of the period the animals spent under conditions of a tropical climate. Conditions of transportation by ship from the splashdown point also may be of considerable significance.

Consequently, the obtained results attest to the fact that a complex of factors of spaceflight, in combination with starvation,

caused changes in the organs of turtles which were mainly of an atrophic character: reduction in the walls of the intestine and in the diameter of the seminiferous tubules, a drop in the volume of liver and kidney cells and in the number of cells of the germinal epithelium of the testes, accumulation of lipofuscin in the organs, suppression of mitotic activity of the epithelium of the intestine mucous membrane and the haemopoietic tissue of the spleen. The enzyme activity of the cells was also changed. Starvation and transportation to the cosmodrome led to less expressed atrophy of the tissues.

Comparison of the changes appearing in the experimental and in the control animals showed that the basic structural changes in the turtles were caused by starvation and to a lesser degree by factors of spaceflight.

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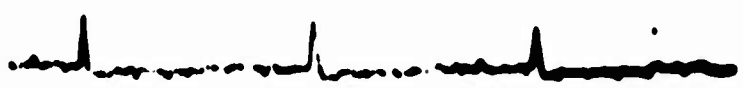
Fig. 1. Turtles No. 22 and No. 37 on board the automatic station "Zond-5" during its flight around the Moon.

(1)



(2)

Fig. 2. Electrocardiograms of turtles (lead 2, paper feed speed 30 mm/s). 1 – Turtle No. 22 (experimental), 2 – Turtle No. 47 (control).

Fig. 3. Intestine. Combined tissue block. The diameter f of the gut and the thickness of the layer are reduced, villi are shortened in turtles of the experimental group (Brashe. mag. lens).

Fig. 4. Intestine of a turtle of the experimental group. In the epithelium of the crypt there are visible cells with hyperchromic nuclei, certain of which "precipitate" into the lumen of the intestine. (Hematoxylin-eosin. Mon. IV, ob. 40, 800×).

Fig. 5.

Fig. 6.

Fig. 5. Intestine. Combined tissue block. In the turtle of the experimental group, the PAS-positive reaction in the depth of the crypt is noticeably weakened (Shabadash. mag. lens).

Fig. 6. Testis of turtle located on the station "Zond-5." Diameter of tubules is reduced, the geminal epithelium is broken up and impoverished of cells. Significant deposits of brown pigment in the interstitial tissue. (Brashe. Mon. VI, ob. 20, mag. 240×).

Fig. 7. Testis of undisturbed turtle. Compare with Fig. 6. (Brashe. Mon. VI, ob. 20, mag. 240×).

Fig. 8. Seminal vesicles of a turtle flown on the "Zond-5" station. Spermatozoids are absent from the lumen. (Brashe. Mon. VI, ob. 16, mag. 115×).

Fig. 9.

Fig. 10.

Fig. 9. Seminal vesicles of undisturbed turtle in the vivarium. Lumina are filled with spermatozoids. (Brashe. Mon. VI, ob. 16, mag. 115×).

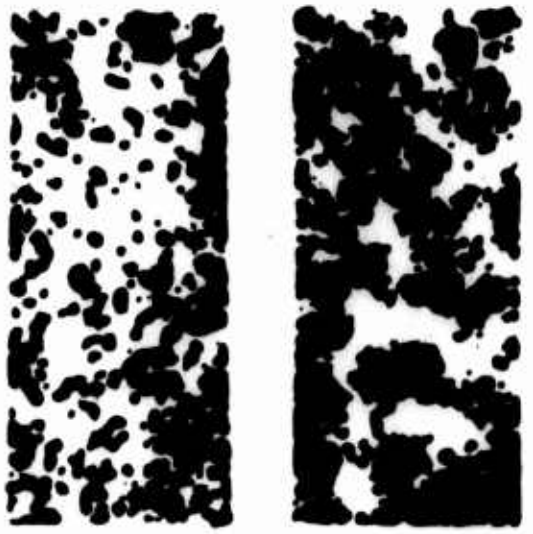
Fig. 10. Liver. Combined tissue block. In turtles flown on the "Zond-5" the dimensions of cells are reduced, their cytoplasm is dark and basophilic, the quantity of pigment is increased. (Hematoxylin-eosin. Mon. VI, ob. 40, mag. 470× .

Fig. 11. Liver of a turtle from the undisturbed group. Glycogen is evident in the cells in a moderate quantity (Shabadash. Mon. VI, ob. 40, mag. 470×).

Fig. 12. Liver of turtle from experimental group. The content of glycogen is increased. Compare with Fig. 11. (Shabadash. Mon. VI, Ob. 40, Mag. 470×).

Fig. 13. Liver. Combined tissue block. In turtles flown on the "Zond-5" there is no fat in the cells. (Scharlach red. Mon. IV, Ob. 40, Mag. 800×).

Fig. 15.



(a) (b)

Fig. 14.

Fig. 14. Liver. Activity of monoaminooxidase in turtles flown on the "Zond-5" is reduced (a) and is significant (b) in the undisturbed animals. (Glenner et al. Mon. VI, Ob. 16, Mag. 115×).

Fig. 15. Seminal vesicles of a turtle located at the cosmodrome. A small quantity of spermatozoids is retained in the lumina. (Brashe. Mon. VI, Ob. 16, Mag. 115×).

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ABSTRACT

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The Zond 5 automatic station carried two steppe turtles on a circumlunar flight. Two turtles were subjected to the same ground transport as the experimental group and four specimens were retained in the vivarium as an undisturbed control sample. Hematological, pathomorphological, histochemical and EKG procedures were used to investigate the biological effects of space flight and ground disturbance on the animals. A process using combined tissue blocks from different animals for histological and histochemical studies is described. Tissue photo micrographs illustrate the findings. The use of the disturbed control group made it possible to separate changes due to spaceflight from those due to transport and caging on the ground. (Fifteen figures are included in the parent document, which is available on microfiche.)